Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 92 (2005) 101–107
Food Chemistry
www.elsevier.com/locate/foodchem

Suppression of acyl migration in enzymatic production of structured lipids through temperature programming

Tiankui Yang, Maj-Britt Fruekilde, Xuebing Xu *

BioCentrum-DTU Building 221, Technical University of Denmark, Lyngby 2800, Denmark

Received 4 May 2004; received in revised form 20 July 2004; accepted 26 July 2004

Abstract

Acyl migration in the glycerol backbone often leads to the increase of by-products in the enzymatic production of specific structured lipids. Acyl migration is a thermodynamic process and is very difficult to stop fully in actual reactions. The objective of this study was to investigate the feasibility of suppressing acyl migration by a programmed change of reaction temperature without loss of reaction yield. The model reactions were the acidolysis of tripalmitin with conjugated linoleic acid (CLA) or with caprylic acid (CA) targeted for human milk fat substitutes. Acyl migration was considerably inhibited in the temperature-programmed acidolysis of PPP with CLA or CA, with only slight reduction of acyl incorporation, the reaction leading to the required products. Acyl migration was reduced by 29% (35 h) and 45% (48 h), respectively, in the acidolysis of PPP with CLA under solvent and solvent-free systems, in comparison with 37% (35 h) and 61% (48 h), respectively, for the acidolysis of PPP and CA. Acyl migration in the acidolysis of PPP with CA was, in general, lower than the acidolysis of PPP with CLA in both systems. Temperature programming was more prominent in solvent-free systems for the reduction of acyl migration. Acyl incorporation was not significantly affected by temperature programming. The study suggests that it is feasible to reduce acyl migration by programmed change of acidolysis temperature without significant loss of reaction yield.

Keywords: Acidolysis; Acyl migration; Conjugated linoleic acid (CLA); Structured lipids; Temperature programming; Tripalmitin

1. Introduction

Structured lipids (SLs) are triacylglycerols (TGs) containing specific fatty acids in designated positions (Gunstone, 1999) and have become of academic and industrial interest because beneficial effects of TGs are closely associated with their structures. Molecular structures of TGs influence their metabolic fate in organisms (digestion and absorption) (Iwasaki & Yamane, 2000). For the synthesis and production of SLs using enzymatic catalysis, wide research work has been conducted in the past 10 years (Akoh & Yee, 1997; Hamam & Shahidi, 2004; Iwasaki, Han, Narita, Rosu, & Yamane, 1999; Quinlan & Chandler, 1992; Schmid, Bornscheuer, Soumanou, McNeill, & Schmid, 1998, 1999; Sharma, Arora, & Wadhwa, 2001; Shimada et al., 1996; Senanayake & Shahidi, 2002; Soumanou, Bornscheuer, & Schmid, 1998a, 1998b; Willis & Marangoni, 1999; Xu, 2000a; Yang & Xu, 2002). Based on structure and fatty acid composition, SLs are often referred to as human milkfat substitutes (HMFS), cocoa butter equivalents (CBE), and MLM-type TGs (M-medium chain and L-long chain fatty acids). MLM-type TGs, containing medium-chain fatty acids at *sn*-1 and *sn*-3 positions and long chain fatty acids at the *sn*-2 position, provide rapid delivery of energy via oxidation of the more hydrophilic medium-chain fatty acids. HMFS are supposed to contain 20–25% palmitic acid predominantly

* Corresponding author. Tel.: +45 45 25 2773; fax: +45 45 88 49 22.
E-mail address: xx@biocentrum.dtu.dk (X. Xu).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.07.007

esterified at the *sn*-2 position, which lead to considerable significance and consequence for the absorption of fat and minerals in infants.

SLs are extremely difficult to prepare by chemical synthesis due to the many purification steps required to remove intermediate products (Soumanou et al., 1998a, 1998b). Another approach is enzyme-catalyzed interesterification, especially *sn*-1,3 specific lipase-catalyzed acidolysis. The advantages of enzymatic reaction lie in its selectivity, mild reaction conditions, little or no unwanted side-reactions or by-products, ease of product recovery, easy control over the process and less waste disposal (Akoh, 1997). The one-step acidolysis reaction route is depicted in Fig. 1. Regiospecificity is an essential requirement for the acidolysis to produce pure SLs. This process is to replace fatty acids (A) specifically at the *sn*-1 and *sn*-3 positions of TG (AAA) with the desired one (B) by a 1,3-specific lipase, leaving the fatty acid (A) at the *sn*-2 position unchanged. Nevertheless, side-reactions (acyl migration) could not be avoided due to the existence of diacylglycerols (DGs), which leads to the formation of undesirable products (*sn*-ABA, *sn*-BBA and *sn*-ABB). Acyl migration generally involves migration from *sn*-1,3 to *sn*-2 positions but also occurs with migration of acyls from the *sn*-2 into the *sn*-1,3 positions. The possible mechanisms of acyl migration have previously been discussed (Xu, 2000b). It is difficult to efficiently separate the desirable product from by-products on an industrial scale. The effect of reaction parameters on acyl migration has been elucidated and temperature is one of the most important parameters that have a profound influence on the migration rate, since acyl migration is a thermodynamic process (Xu, 2000b). There have been no possible methods to fully stop the acyl migration, but minimization of acyl migration can be expected under optimized conditions.

In a "two-step" process for the production of pure SLs, alcoholysis was the first step and esterification was the second step. The "two-step" process efficiently suppressed acyl migration in both alcoholysis and esterification steps in a solvent system at low temperatures (Iwasaki & Yamane, 2000; Schmid, Bornscheuer, Soumanou, McNeill, & Schmid, 1998, 1999; Soumanou et al., 1998a, 1998b).

From our evaluation, we believe that temperature plays a very important role in the increase of acyl migration. On the other hand, temperature also affects the reaction activity of enzymes, in general, according to the Arrhenius law. For lipase-catalyzed acidolysis, we found that temperature had a stronger impact on migration than on incorporation, especially with the immobilized lipases tested (Xu, 1998). This provides a positive clue to apply a lower temperature for lipase-catalyzed acidolysis.

The reaction yield is very much based on the reaction equilibrium that can be reached. In the main reaction of Fig. 1, A is a released fatty acid from the reaction. If A can be removed from the reaction system, the reaction equilibrium will be broken and driven further to the product side. On consideration of low temperature for lipase-catalyzed acidolysis, we would expect A to be "frozen" and not actively participate in the reaction. This can be achieved by using a fatty acid with a higher melting point. This led to selection of the model system with tripalmitin as the substrate for production of HMFS. Palmitic acid has a melting point of 63 °C, much higher than caprylic acid or CLA as acyl donors. This would not, therefore, severely affect the reactivity of acyl donors at a lower temperature. Thus, lower temperature will reduce the enzyme activity and substrate mass transfer in the solvent-free system, but will push a higher reaction yield through the breaking of the reaction equilibrium by "inactivating" the released fatty acid. We expect that the former effect can be compensated by the latter, so that acyl migration can be eventually suppressed without the loss of reaction yield.

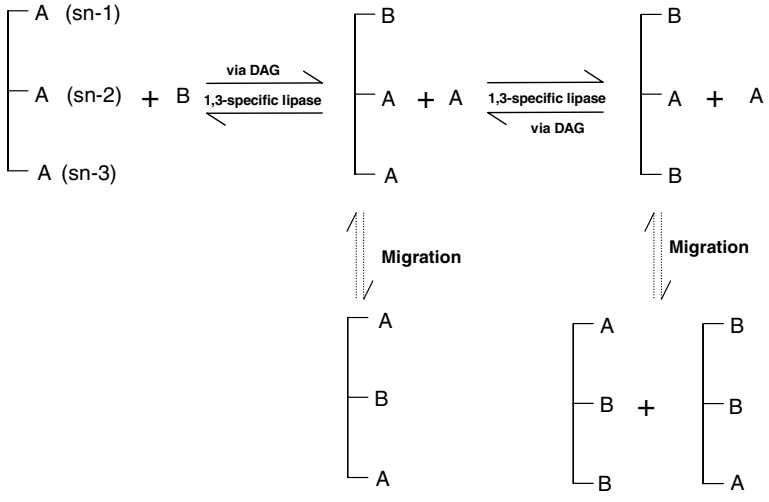

Fig. 1. Scheme of acidolysis catalyzed by *sn*-1,3 specific lipase. A and B stand for acyl groups. DAG is diacylglycerol.